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Abstract

BACKGROUND: The demand for zero and reduced-sugar food products containing cocoa is expanding continuously. The present study was designed to evaluate the feasibility of producing high-quality chocolate sweetened with a crude extract of *Stevia rebaudiana* (Bertoni) prepared by a green microwave-assisted water-steam extraction procedure. Seven approximately isosweet chocolate formulations were developed, mixing cocoa paste, sucrose, commercial stevioside, crude green extract and maltitol in different proportions. All samples were analyzed for the determination of polyphenol and flavonoid content, antioxidant activity, and sensory acceptability.

RESULTS: The use of a crude stevia extract allowed low-sugar, high-quality chocolates to be obtained that were also acceptable by consumers and had a significant increased antioxidant activity. Moreover, consumers' segmentation revealed a cluster of consumers showing the same overall liking for the sample with 50% sucrose replaced by the stevia crude extract as that obtained with the commercial stevioside and the control sample (without sucrose replacement).

CONCLUSION: The results provide information that can contribute to promoting the development of sweet food products, with advantages in terms of an improved nutritional value (reduced sugar content and increased antioxidant activity) and a reduced impact of the production process on the environment.

Keywords: Chocolate**;** Stevia; green process; microwave-assisted steam extraction; reduced sugar content; consumer acceptability.

1. Introduction

Chocolate consumption increased constantly in Western countries and since 1990 a second global boom driven by emerging markets took place; the forecasts for chocolate are for rising demand (for a deep economical analysis see Squicciarini & Swinnen 2016). Chocolate's health benefits have been reported since its early use by Aztec and Maya populations; recently several scientific studies have focussed on cocoa and chocolate bioactive molecules that can be of value to many aspects of health (for an overview Ellam & Williamson 2013; Watson, Preedy & Zibadi 2013). In 2012 the European Food Safety Agency allowed a health claim for dark chocolate with high flavanol content (EFSA 2012). Beside cocoa, most chocolate based snacks contain additional calories in large part due to the content of sucrose, which contributes to the daily sucrose intake of consumers. Increasing health consciousness and proof that overweight and obesity contribute to a large proportion of noncommunicable diseases, make the demand for zero and reduced-sugar food products containing cocoa expanding continuously. The addition of sucrose in chocolate makes the cocoa less bitter, improves smoothness and overall palatability, but also provides bulk and reduces water activity. Some studies have been conducted to formulate reduced calorie chocolate using different mixtures of sweeteners and bulking agents (Belšcˇak-Cvitanovic´ et al. 2015, Aidoo, Depypere, Afoakw, & Dewettinck, 2013; Shah, Jones, & Vasiljevic, 2010).

Stevia rebaudiana Bert., a plant native to Paraguay, has a long history of use as a sweetener and in the treatment of several diseases (Kinghorn, 2002; Yadav & Guleria 2012; Lemus-Mondaca, Vega-Galvez, Zura-Bravo & Ah-Hen 2012). Its sweetness is mainly due to a group of structurally-related compounds, steviol glycosides, which present a common aglycone, known as steviol (*ent*-13-hydroxykaur-16-en-18-oic acid), and differ in the number and types of sugar residues. Stevioside is the most abundant steviol glycoside and has been reported to be between 210 and 300 times sweeter than sucrose (Crammer & Ikan 1987; Kinghorn & Soejato 1986). As for other high-potency sweeteners (DuBois & Prakash 2012), steviol glycosides present bitter and off-tastes (Kinghorn & Soejato 1986, Prakash, DuBois, Clos, Wilkens & Fosdick 2008), as well as consumer differences in sweeteners sensitivity and acceptance, suggesting that most of the variation in sensitivity may have a genetic basis (Simons et al 2008). Hofmann, Hellfritsch, Brockhoff, Stähler, & Meyerhof (2012) have identified hTAS2R4 and hTAS2R14 as the receptors that mediate the bitter off-taste of steviol glycosides in vitro, while Risso & al. (2014) have proven that polymorphisms in these two bitter receptors are functional for stevioside bitterness perception. Beside sweetness, steviol glycosides present other bioactivities in humans, including antihyperglycaemic, antihypertensive and anticancer activity (Brahmachari, Mandal, Roy, Mondal & Brahmachari 2011; Yadav & Guleria 2012).

The use of at least 95% purified steviol glycosides as sweeteners was authorized in 2008 in USA and in 2011 in the EU, becoming the first high potency sweeteners of natural origin on the market. This fact has greatly contributed to the growing demand of these sweeteners and a report by Leatherhead Food Research & Mintel (2013) forecasts the value of steviol glycosides as an additive for use in food and beverage manufacture to further grow from $110 million USD in 2013, to $275 million USD by 2017. Some of the most common soft drinks are now also available on the market in the version sweetened with steviol glycosides.

In addition to steviol glycosides, Stevia leaves also contain phenolic compounds, flavonoids and other antioxidants with potential beneficial effects on human health (Lemus-Mondaca, et al. 2012, Kim, Yang, Lee & Kang, 2011). In particular has been proven that the crude aqueous

extracts from S. rebaudiana can exert a cellular scavenging activity against free radicals (Bender, Graziano & Zimmermann 2015).

To date, the use of crude stevia extracts has not been approved for use as a food or as additive in USA and EU, but application for novel food authorisation for dried stevia leaves has been submitted.

Another important research topic in recent years has been the design of more efficient extraction processes that may address the requirements of process intensification and energy saving. Safety, sustainability, environmental and economic factors are all forcing industries to turn to non-conventional technologies and greener protocols (Chemat et al., 2012). Microwave-assisted extraction (MAE) is today a conceivable reality beyond lab-scale procedures (Chemat & Cravotto, 2013; Filly et al. 2014) and recognized as one of the most efficient, eco-friendly extraction method. Dried leaves of *Stevia rebaudiana* B. have been extracted by classic and non-conventional methods such as MAE (Jaitak, Bandna & Kaul, 2009) and ultrasound-assisted extraction (Liu, Li & Tang, 2010) generally using methanol, ethanol and water as single solvents as well as in binary mixtures. Supercritical and subcritical CO_2 extraction (Liu, Ong, & Li 1997) has also been performed via the addition of polar organic solvents such as methanol or acetonitrile. The Accelerated Solvent Extraction (water at 100°C) of stevia leaf powder has recently been optimized in a response surface methodology study to investigate the influence of temperature, static time and cycles number on extraction yields (Jentzer, Alignan, Vaca-Garcia, Rigal & Vilarem, 2015).

A lab-scale green approach to stevia extraction included two batch systems: a pressurized hot water extraction (PHWE) and a pressurized hot water MAE (Teo, Tan, Yong, Hew & Ong 2009). The latter highlights the beneficial role of microwave irradiation, showed higher extraction efficiency with shorter extraction time, although the 1:100 plant/water ratio could only be applied for analytical purposes. MAE cannot be used to extract dry plant material or in general samples in which the moisture content is low. To overcome this restriction, microwave-assisted steam distillation (Numata, Yarita, Aoyagi, & Takatsu, 2003) and

microwave-assisted water steam extraction (MAWSE) (Song et al., 2012; Song et al., 2014) were developed for analytical applications. These extraction methods were efficient, fast and enable a lower organic solvents consumption. A successful process for stevia extraction by a direct water steam injection into the extractor was described by Kumar et al. (2006).

This study has been designed to evaluate the production feasibility of high-quality reduced sugar chocolate using a crude green extract of *Stevia rebaudiana* B. as sweetener. Cocoa paste, sucrose, commercial stevioside, maltitol and a crude green extract of *Stevia rebaudiana* B. in different proportions have been used to obtain 7 isosweet chocolate sample, then analysed for their polyphenols and flavonoids content, antioxidant activity (ORAC, Oxygen Radical Absorbance Capacity) and sensory acceptability by means of a consumer test.

2. Materials and methods

2.1. Chemicals

Stevioside sweetener was supplied by Nastevia, Stevia Italia s.r.l., Italy; stevia leaves were kindly supplied by CONSULT AZIONE S.A.S, Turin, Italy.

Acetone (analytical grade) was purchased from VWR International Inc. (West Chester, PA). Folin-Ciocalteu's reagent, fluorescein sodium salt, 6-hydroxy-2,5,7,8-tetramethyl-2-carboxylic acid (Trolox), were obtained from Sigma-Aldrich Inc. (St.Louis, MO). 2,2'-azobis(2-amidinopropane)dihydrochloride (AAPH) was obtained from Polysciences Inc. (Warrington, PA). All other reagents were from Carlo Erba, Milan, Italy.

2.2 Green extraction of Stevia rebaudiana B.

The microwave-assisted water steam extraction of stevia leaves, previously cut into small pieces (4-7 mm), was carried out at atmospheric pressure in the NEOS-GR, a dedicated microwave reactor (Milestone srl, Bergamo IT) (Fig. 1). The reactor chamber was directly connected from the top with the steam generator Vaporetto ECOPRO 3000 (Polti, Como, IT)

and from the bottom to a condenser and a round bottomed Pyrex flask equipped with an open gastap. The steam generator (2 kW, max pressure 4 bar) produced a vapour flow of 100 g/min. The optimized extraction was carried out on 500 g plant material, subjected to a steam flow for 13 min. After 1 min, the swollen leaves were irradiated with microwaves (800 W) in an on/off sequence (2 min cycle) for a total of 12 min. Compared with any other extraction method in water, MAWSE produce a much more concentrated extract (250 mL ± 8% from 500 g leaves), very little water to evaporate (20 - 60 times lower) and high energy savings. The condensed aqueous extract collected from the bottom of the reactor was freeze dried and gave a brown extract powder (1.4 g ± 3%).

2.3 Relative Sweetness (RS) of the stevia green extract and of stevioside

A 3% w/v sucrose standard solution in distilled water was prepared and its sweetness compared by an untrained panel of 6 people to a 0.5% w/v crude stevia extract solution in distilled water or a 0.2% w/v commercial stevioside; the solution of the sample to be rated was diluted until considered isosweet to the 3% sucrose standard solution.

The relative sweetnesses, RS, defined as RS = [sucrose]/$[X]_{isosweet}$. were calculated as:

RS crude stevia extract = 50; RS commercial stevioside = 220.

For maltitol a RS of 0,8 has been used.

2.4 Chocolate sample preparation

For the production of all chocolate samples, the laboratories, expertise and raw material of a high quality chocolate producer based in Italy were used.

The standard recipe for a 70% dark chocolate using only cocoa paste and sugar as ingredients from the hosting producer was used. The amount of commercial stevioside and/or of crude extract were calculated in order to obtain 7 approximately equally sweet final products, in which part or all the sucrose was substituted. The same cocoa paste were used for all the samples and no emulsifiers were added.

Isosweet chocolate sample composition:

- Standard recipe (STD): 2000 g of cocoa paste and 600 g of sucrose.

- Sucrose completely substituted with commercial stevioside (**100S**): 2000 g of cocoa paste and 2.8 g of commercial stevioside.

- 50% of sucrose substituted with commercial stevioside (**50S**): 2000 g of cocoa paste, 300 g of sucrose and 1.4 g of steviol glycosides.

-sucrose completely substituted, 50% with maltitol and 50% with commercial stevioside (**50M-50S**): 2000 g of cocoa paste, 360 g of maltitol and 1.4 g of steviol glycosides.

-sucrose completely substituted with crude extract (**100E**): 2000 g of cocoa paste and 12 g of green extract.

-50% of sucrose substituted with crude extract (**50E**): 2000 g of cocoa paste, 300 g of sucrose and 6 g of green extract.

-sucrose completely substituted, 50% with maltitol and 50% with crude extract (**50M-50E**): 2000 g of cocoa paste, 360 g of maltitol and 6 g of green extract.

The cocoa paste was put into a small scale pebble mill and ground for 10 minutes, then the sweetening agents were added and the mixture blended for 30 minutes. The obtained liquid chocolate was tempered in the same conditions and filled into square shaped forms of 2 cm x 2 cm, and solidified at 12 °C.

The obtained chocolate squares were packed in sealed aluminium bags and stored in a cool and dry place.

Once emptied, the pebble mill was cleaned with cocoa butter in order to avoid contamination with the following sample.

2.5 Determination of polyphenols, flavonoids and antioxidant activity

The extraction of polyphenols from chocolate samples and stevia crude extract has been performed by slightly modifying a previously reported method (Ninfali & Bacchiocca, 2003).

2.5.1 Preparation of chocolate extracts for bioactive parameters determination

0.45 g of grated chocolate were added to 6.65 mL of 70% acetone and stirred for 60 min at 20 °C, then centrifuged for 5 min at 3000 rpm. The supernatant was collected and the solid washed two times with 4.5 mL of 70% acetone, stirred for 15 min and centrifuged for 15 min at 3000 rpm. The solid was re-suspended in 4.5 mL acidified water and sonicated for 2 min, then centrifuged at 3000 rpm for 5 min. The four supernatants were combined (measuring the total volume) and used for the analysis of total phenols, flavonoids and antioxidant activity (ORAC).

2.5.2 Preparation of stevia crude extract for bioactive parameters determination

To 0.5 g of crude stevia extract, 25 mL of 50% acetone were added, and the mixture vortexed for 5 min and centrifuged for 10 min at 3000 rpm. The supernatant was collected and the solid re-suspended with 5.5 mL of 5% perchloric acid, vortexed for 5 min and centrifuged for 10 min at 3000 rpm. The procedure was repeated; the three supernatants were combined, the total volume measured and the sample used for the analysis of total phenols, flavonoids and antioxidant activity (ORAC).

2.5.3 Antioxidant Capacity

Antioxidant activity measurements were carried out by the ORAC assay (Oxygen Radical Absorbance Capacity), according to Ninfali & Bacchiocca, 2003, on a Fluostar Optima plate reader fluorimeter (BMG Labtech, Offenburgh, Germany) equipped with a temperature-controlled incubation chamber and automatic injection pump. Incubator temperature was set at 37 °C. The reaction mixture for the assay was as follows: 200 µL of 0.096 µM fluorescein sodium salt in 0.075 M Na-phosphate buffer (pH 7.0), and 20 µL of sample or Trolox

(standard). A calibration curve was made each time with the standard Trolox (500, 300, 100, 50 and 25 µM). The blank was 0.075 M Na-phosphate buffer (pH 7.0). The reaction was initiated with 40 µL of 0.33 M 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). Fluorescence was read at 485 nm ex. and 520 nm em. until complete extinction. ORAC values were expressed as µmol Trolox Equivalents (TE)/g.

2.5.4 Total Phenol and Flavonoid Assays

Phenols were assayed according to the Folin-Ciocolteau method (Singleton, Orthofer & Lamuela-Raventos, 1999) and results were expressed as mg Caffeic Acid Equivalents (CAE). Flavonoids were determined by the method of Eberhardt, Lee, & Liu, (2000).

2.6. Sensory evaluation

In order to assess the sensory acceptability of chocolate samples a consumer test was conducted. Ninety-five regular consumers of chocolate (39% males, 61% females, ranging from 18 to 69 years, with a mean age of 27) participated in this study. They had seen or received an invitation and volunteered based on their interest and availability. Written informed consent was obtained from each subject after the experiment was described to them. All tests were conducted individually and social interaction was not permitted. The experimenter verbally introduced the consumers to the data collection procedure. The samples (10 g) were served in blind conditions, in clear plastic cup (96 mL) hermetically sealed with a clear plastic lid and coded with a random three-digit number. Samples were served in completely randomized and balanced order among subjects. Samples were evaluated at room temperature (20±1 °C). Subjects were instructed to observe, smell and taste the samples and to rate their liking for appearance, odour, taste, flavour, texture and overall liking on a 9-point hedonic scale ranging from 'dislike extremely' (1) to 'like extremely' (9) (Peryam and Pilgrim, 1957). Participants were required to rinse their mouth with still water for about one minute

before beginning the test and between samples. Consumers took between 15 and 20 minutes to complete their evaluation.

2.7 Statistical analysis.

Polyphenol and flavonoid concentrations were measured in triplicate and results were expressed as the mean ± SD of three values. Data of antioxidant capacity were performed by 6 independent determinations for each sample and results were the mean ± SD of 6 values. Statistical significance was tested using Student's *t* test with a $p<0.05$ indicating a significant difference between data sets.

Liking data (for appearance, odour, taste, flavour, texture, overall) from consumers were independently submitted to a two-way ANOVA mixed model (fixed factor: sample; random factor: subject) by performing LSD ($p < 0.05$). Moreover, liking data were submitted to a 2-way ANOVA fixed model assuming sample and cluster as main effects. Overall liking ratings expressed by all 95 subjects were analyzed by means of Principal Component Analysis (PCA) in order to obtain an Internal Preference Map (IMP) for explorative purposes. To investigate potential segments of consumers with different chocolate preferences, the broken stick criteria (Todeschini, 1998) was used, whereby the first four principal components were selected to limit overloaded information and noise implied in components with low variance and analyzed by cluster analysis applying an Euclidean distance metric and a Ward method of linkage. Two groups of consumers were defined. Liking data from each cluster were independently submitted to a 2-way ANOVA mixed model (fixed factor: product; random factor; subject), with LDS ($p \leq 0.05$). Liking ratings were analysed using Systat version 13.1 (Systat Software Inc, USA) and The Unscrambler X version 10.3 (Camo Software AS, Norway) softwares.

3. Results and Discussion

3.1 Phenols and antioxidant capacity of chocolates and ingredients

The content of phenols, flavonoids and ORAC per g of each chocolate sample, as well as of the crude green extract of stevia, commercial stevioside and maltitol are shown in **Table 1.** Differences among the chocolate samples were evidenced by the ORAC value, due to the higher sensitivity of this assay with respect to Folin Ciocolteau or the $AlCl_3$ assays. As sucrose does not express any significant contribution to the ORAC value (data not shown), the standard recipe STD with 24% sucrose, provides the antioxidant capacity of the cocoa's own phenolic compounds, i.e phenolic acids and flavonoids (Belšc˘ak-Cvitanovic´ et al. 2015). With regards to the other chocolate samples, the highest ORAC value was obtained in the chocolate sample **100S,** in which all the sucrose was replaced with stevioside, followed by the sample **100E**, in which all the sucrose was replaced with stevia crude extract. From the comparative analysis versus the **STD** chocolate, an ORAC increase of 51 and 36% was measured in samples **100S** and **100E** respectively. Half a dose of stevioside also provided a statistically significant increase in the ORAC value of the sample **50S**, but this did not occur in the **50E** sample, in which half a dose of the stevia crude extract was used. In comparison with the **STD**, the sample **50M-50S** showed a significant increase in ORAC value, as well as the sample **50M-50E**.

Table 1 also shows the ORAC/Phenols ratio, which indicates the antioxidant activity of one mg of phenols contained in the sample. All samples are clustered between 8.4 and 11.80 ORAC/Phenols ratio, except the pure stevioside which reached the value of 60.57).

3.2 Consumers' preferences

The Internal Preference Map obtained from a PCA on overall liking data of 95 consumers for the seven tested chocolates is reported in **Figure 2**. The first two components accounted for 58% of the total variation (PC1: 43% and PC2: 15%). Samples are mainly discriminated along PC1 according to the percentage of sucrose substitution, with the **STD** chocolate on the right part of the map and the chocolates with 100% sucrose substitution on the opposite side. PC2 distinguished the samples as a function of the presence or absence of maltitol in the chocolate

recipe. Most of the consumers' preferences were observed for the **STD** chocolate and both for samples with 50% of sucrose substituted with the stevia crude extract (**50E**) or commercial stevioside (**50S**). On the other hand, it is evident that samples with 100% of sucrose substituted with the stevia crude extract or commercial stevioside did not meet the consumers' preference (**100E** and **100S**).

The average liking ratings for each chocolate sample expressed by the totality of consumers are shown in **Table 2**. The highest overall liking value was observed for the **STD** sample. Nevertheless, satisfying average liking ratings were obtained for the samples with 50% of sucrose substituted with the stevia crude extract (**50E**) or commercial stevioside (**50S**), assessed higher than the central value of the scale (5 = neither like nor dislike), considered as the minimum acceptable value. It is worth noting that these samples are not significantly different, independently from their specific recipe. Contrariwise samples **100S** and **100E**, in which all the sucrose was replaced with commercial stevioside or crude stevia extract respectively, were not considered acceptable by consumers. The slight dislike observed for these two chocolates was probably due to their low performance in terms of taste, flavour and texture, which were significantly lower than that for the other samples. No differences in liking for odour were noticed across the seven samples.

Applying the cluster analysis to the overall liking data, two segments of consumers were obtained: the first consisting of 59 subjects (62%), namely Cluster 1; the second consisting of 36 subjects (38%), namely Cluster 2. No significant differences were found between the two clusters considering the gender, the age, the educational level and most of the investigated Food Choice Questionnaire items ($p>0.05$). On the contrary, significant differences were noticed between the two segments regarding the importance given to the items related to the sensory appeal of food eaten on a typical day. In particular, Cluster 2 considered the items "looks nice" (Cluster 1: 3.20; Cluster 2: 3.75; $p=0.019$), "tastes good" (Cluster 1: 4.53; Cluster 2: 4.83; $p=0.006$), and "has a pleasant texture" (Cluster 1: 3.86; Cluster 2: 4.31; $p=0.007$) more important than Cluster 1.

The results of the 2-way ANOVA fixed model revealed a significant effect in the interaction cluster*product, with Cluster 1 rating the overall liking for most of the products (except for **100S** and **50E**) higher than Cluster 2.

In order to graphically visualize the preferences of the two segments of consumers, the average overall liking ratings obtained by Cluster 1 and Cluster 2 were superimposed on the IMP (**Figure 2**). Even if both clusters liked the **STD** samples better, an interesting difference was observed for samples with 50% of sucrose substitution: Cluster 2 showed a preference for samples obtained substituting 50% of sucrose with crude extract (**50E**) or commercial stevioside (**50S**), while Cluster 1 preferred the chocolates with sucrose completely substituted, 50% with maltitol and 50% with crude extract **(50M-50E),** or 50% with maltitol and 50% with commercial stevioside (and **50M-50S**). To better investigate these different preference tendencies, the results of the 2-way ANOVA mixed model independently conducted on clusters' data and the average ratings for liking expressed by the two clusters of consumers for appearance, aroma, taste, flavour, texture and overall liking of the seven tested chocolates are reported in **Table 2**. In terms of overall liking, taste, flavour and texture, Cluster 1 rated the **STD** chocolate as highly acceptable and the samples **50S**, **50M-50S**, **50E** and **50M-50E** as acceptable. Cluster 1 did not discriminate between samples neither in terms of appearance nor for odour. Cluster 2 liked the samples **50S**, **50E** and **STD** the most. No differences in terms of overall liking, appearance, taste, flavour and texture were noticed between the samples with 50% of sucrose substituted with commercial stevioside (**50S**) or stevia crude extract (**50E**) and the **STD** sample. Lower ratings were given by Cluster 2 to the other products, in particular to sample **100E** which was the least liked chocolate. In general, higher discrimination ability was observed in Cluster 2 than Cluster 1. This difference may be partially influenced, as already mentioned, by the higher importance for sensory appeal of food declared by Cluster 2. It could be hypothesized that Cluster 2 paid higher attention to the sensory properties of samples than Cluster 1 during the liking test, thus it was more able to detect subtle sensory differences in the chocolate samples.

4. Conclusions

Steviol glycosides are to date the only high-potency sweeteners of natural origin available on the market in Europe, whilst the use of crude stevia extract is not yet allowed. Several studies reported that the whole plant provided potential beneficial effects on human health. In this paper we proved the production feasibility of high-quality chocolate using a "green" crude stevia extract obtained with a microwave-assisted water-steam extraction procedure. We produced seven isosweet chocolate samples in which part or all the sucrose was substituted with commercial stevioside or with our green crude extract. We compared the acceptability of the seven chocolate samples by means of a consumer test. The results indicate that the samples with 50% sucrose substituted with the stevia crude extract (**50E**) present the same overall liking (5.84±0.17) as those obtained with 50% sucrose substituted with the commercial purified stevioside (**50S**, 5.85 ±0.16), being both above the considered minimum acceptable value of 5. Polyphenol and flavonoid content, as well as the antioxidant activity of the two samples are comparable, which also proves that the use of a crude green stevia extract to produce a reduced-calorie high quality chocolate is feasible without affecting the total antioxidant activity. The advantage of using the crude extract obtained by applying green technology is relevant to make the whole production faster, cheaper and overall more sustainable. This is especially true for modern, conscious consumers, who are happy to pay for a high-quality product, but who are ever more interested in the impact of the whole production chain.

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Table 1. Concentration of phenols, flavonoids and ORAC values in chocolate samples, crude extract of *stevia*, stevioside and maltitol.

Samples	Phenols mg/g*	Flavonoids mg/g*	ORAC µMol TE/g**	ORAC /Phenols µMol TE/mg PF
STD	27.45±5.05 [a]	19.41±2.92 [a]	243.42±11.95[a]	8.86
50S	25.70±2.93 [a]	19.68±2.95 [a]	282.78±19.49 [bc]	11.00
50M-50E	27.66± 1.14 [a]	19.44±2.93 [a]	266.88±16.24 [b]	9.61
50M-50S	27.04± 3.47 [a]	19.33±2.87 [a]	292.16±17.55[e]	10.80
100E	31.49±2.71 [a]	20.66±3.12 [a]	329.20±28.54[d]	10.45
50E	28.16± 2.24 [a]	19.41±2.92 [a]	236.09±10.4[a]	8.38
100S	30.92±2.85 [a]	22.88±3.41 [a]	365.12±6.26[e]	11.80
Stevia crude extract	76.59±6.82 [b]	34.27±5.14 [b]	647.76±9.18[f]	8.45
STEVIOSIDE	0.84±0.06 [c]	0.42±0.06 [b]	50.88±1.53[g]	60.57
MALTITOL	0	0.32±0.04 [c]	1.31±0.22[h]	N.D.

* Values are the mean ± SD of 3 independent determinations.

** Values are the mean ± SD of 6 independent determinations.

Values in the same column with differing superscripts are significantly different by Student's t test ($p<0.05$).

Figure 1. Microwave-assisted water steam extraction of stevia leaves.

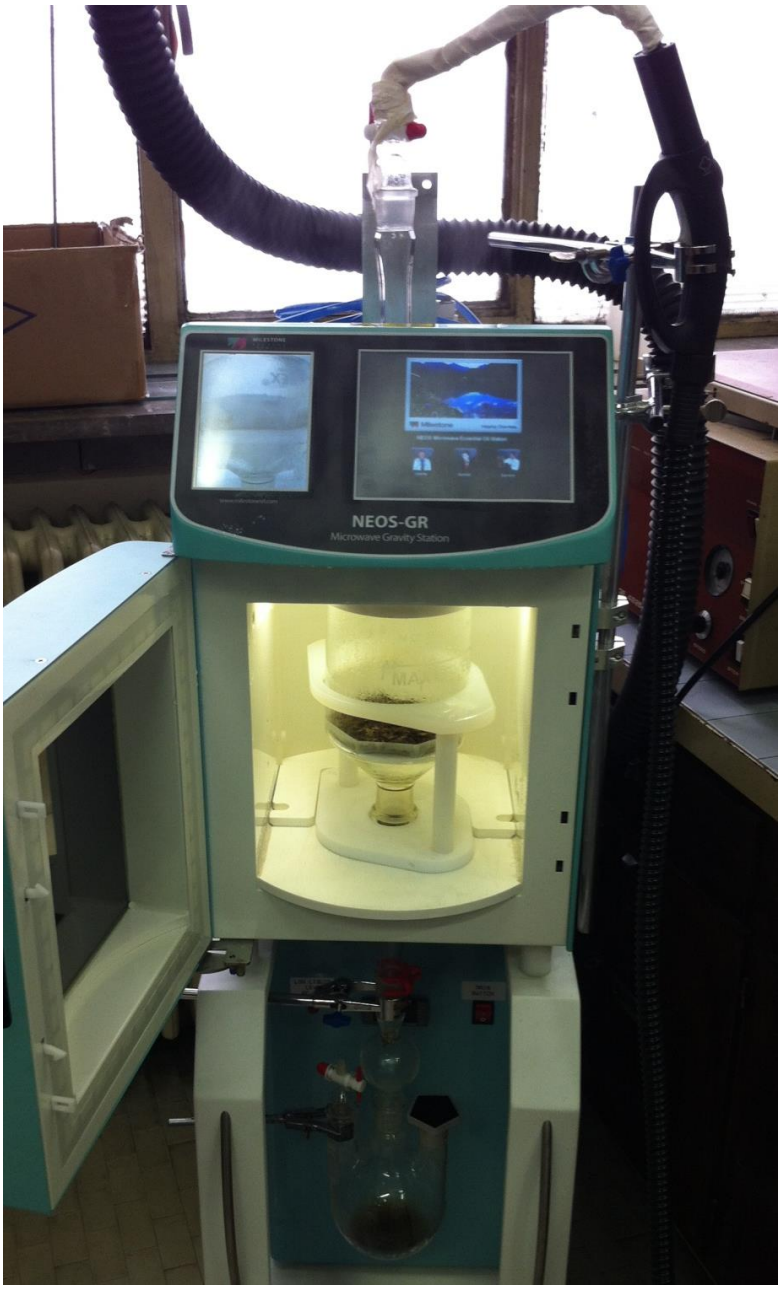
NEOS-GR
Microwave Gravity Station

Figure 2. Internal Preference Map of 95 consumers for the chocolate samples. The average overall liking data from the two identified clusters of subjects (Cluster 1: n=59; Cluster 2: n=36) are superimposed.

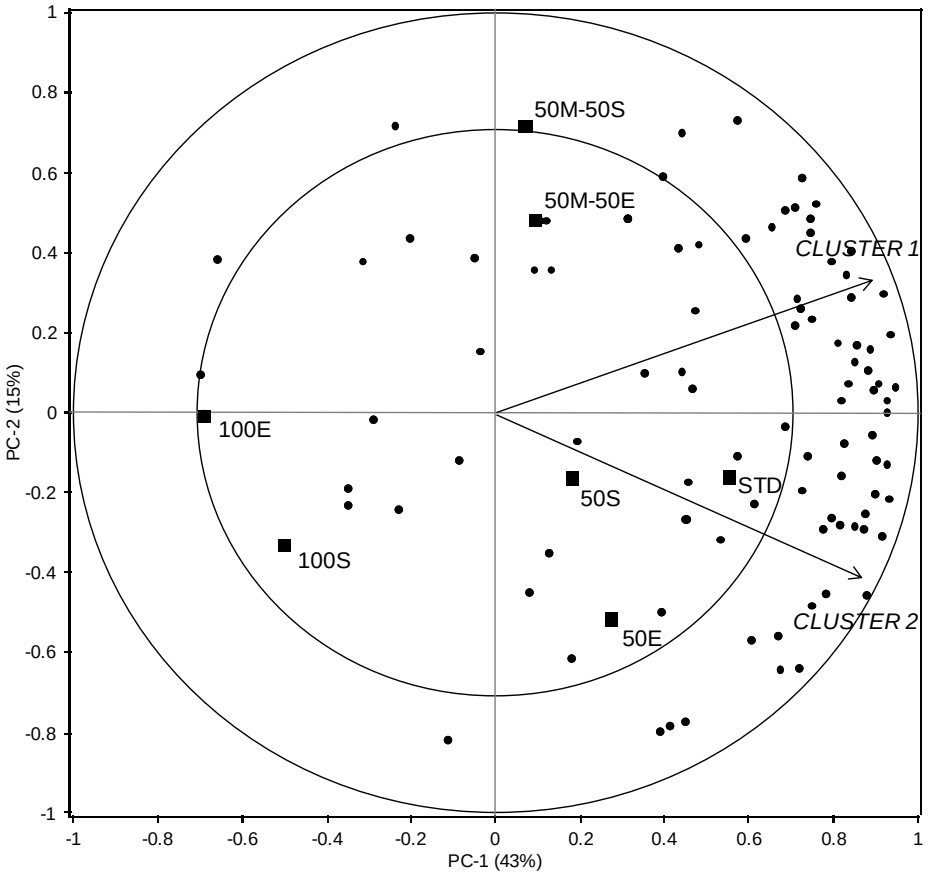

Table 2. Overall liking and liking for appearance, odour, taste, flavour and texture expressed by all consumers (n=95), Cluster 1 (n=59) and Cluster 2 (n=36) for the chocolate samples.

	Overall	Appearance	Odour	Taste	Flavour	Texture
All consumers						
STD	6.35 ± 0.16 ^{a}	6.52 ± 0.16 ^{bc}	6.17 ± 0.17	6.17 ± 0.18 ^{a}	5.99 ± 0.19 ^{a}	6.16 ± 0.19 ^{a}
100S	4.46 ± 0.17 ^{c}	6.78 ± 0.15 ^{a}	6.01 ± 0.17	3.93 ± 0.20 ^{e}	3.85 ± 0.22 ^{c}	5.00 ± 0.22 ^{d}
50S	5.85 ± 0.16 ^{b}	6.74 ± 0.15 ^{ab}	6.20 ± 0.16	5.74 ± 0.19 ^{b}	5.66 ± 0.18 ^{a}	5.92 ± 0.20 ^{ab}
50M-50S	5.58 ± 0.20 ^{b}	6.36 ± 0.16 ^{c}	6.07 ± 0.16	5.28 ± 0.21 ^{cd}	5.12 ± 0.20 ^{b}	5.48 ± 0.20 ^{c}
100E	4.24 ± 0.16 ^{c}	6.54 ± 0.15 ^{abc}	6.06 ± 0.19	3.40 ± 0.21 ^{f}	3.54 ± 0.21 ^{c}	4.96 ± 0.21 ^{d}
50E	5.84 ± 0.17 ^{b}	6.58 ± 0.15 ^{abc}	6.24 ± 0.16	5.67 ± 0.18 ^{bc}	5.62 ± 0.20 ^{a}	5.91 ± 0.19 ^{ab}
50M-50E	5.52 ± 0.20 ^{b}	6.68 ± 0.15 ^{ab}	6.25 ± 0.16	5.20 ± 0.18 ^{d}	5.20 ± 0.18 ^{b}	5.75 ± 0.19 ^{bc}
F	37.74	2.45	0.48	50.53	44.93	10.95
p	<0.0001	0.024	0.826	<0.0001	<0.0001	<0.0001
Cluster 1						
STD	6.53 ± 0.17 ^{a}	6.64 ± 0.19	6.31 ± 0.22	6.31 ± 0.23 ^{a}	6.20 ± 0.22 ^{a}	6.12 ± 0.24 ^{a}
100S	4.19 ± 0.24 ^{d}	6.78 ± 0.20	5.83 ± 0.22	3.70 ± 0.26 ^{c}	3.63 ± 0.26 ^{c}	4.85 ± 0.28 ^{b}
50S	5.88 ± 0.19 ^{bc}	6.73 ± 0.20	6.31 ± 0.20	5.71 ± 0.21 ^{b}	5.66 ± 0.21 ^{b}	5.93 ± 0.25 ^{a}
50M-50S	6.07 ± 0.19 ^{b}	6.49 ± 0.21	6.36 ± 0.19	5.73 ± 0.25 ^{b}	5.50 ± 0.24 ^{b}	5.68 ± 0.25 ^{a}
100E	4.58 ± 0.27 ^{d}	6.66 ± 0.19	6.17 ± 0.23	3.55 ± 0.27 ^{c}	3.55 ± 0.28 ^{c}	5.07 ± 0.28 ^{b}
50E	5.61 ± 0.20 ^{c}	6.59 ± 0.20	6.29 ± 0.21	5.50 ± 0.22 ^{b}	5.44 ± 0.24 ^{b}	5.78 ± 0.24 ^{a}
50M-50E	5.72 ± 0.18 ^{bc}	6.73 ± 0.18	6.47 ± 0.16	5.42 ± 0.22 ^{b}	5.24 ± 0.23 ^{b}	5.75 ± 0.23 ^{a}
F	31.39	0.79	1.53	39.00	34.11	6.64
p	<0.0001	0.575	0.169	<0.0001	<0.0001	<0.0001
Cluster 2						
STD	6.03 ± 0.30 ^{a}	6.31 ± 0.27 ^{bc}	5.94 ± 0.27	5.94 ± 0.31 ^{a}	5.63 ± 0.33 ^{ab}	6.22 ± 0.31 ^{a}
100S	4.92 ± 0.35 ^{b}	6.78 ± 0.22 ^{a}	6.31 ± 0.25	4.31 ± 0.33 ^{b}	4.22 ± 0.40 ^{c}	5.25 ± 0.38 ^{bc}
50S	5.81 ± 0.30 ^{a}	6.75 ± 0.24 ^{ab}	6.03 ± 0.26	5.78 ± 0.35 ^{a}	5.67 ± 0.33 ^{ab}	5.89 ± 0.34 ^{a}
50M-50S	4.78 ± 0.28 ^{b}	6.14 ± 0.27 ^{c}	5.61 ± 0.29	4.51 ± 0.33 ^{b}	4.50 ± 0.33 ^{c}	5.14 ± 0.33 ^{bc}
100E	3.69 ± 0.29 ^{c}	6.33 ± 0.24 ^{abc}	5.89 ± 0.33	3.14 ± 0.32 ^{c}	3.53 ± 0.31 ^{d}	4.77 ± 0.33 ^{c}
50E	6.22 ± 0.28 ^{a}	6.56 ± 0.25 ^{abc}	6.17 ± 0.27	5.94 ± 0.31 ^{a}	5.92 ± 0.34 ^{a}	6.11 ± 0.31 ^{a}
50M-50E	5.18 ± 0.28 ^{b}	6.50 ± 0.29 ^{abc}	5.89 ± 0.33	4.82 ± 0.31 ^{b}	5.14 ± 0.31 ^{b}	5.75 ± 0.33 ^{ab}
F	20.37	2.16	1.03	20.06	15.50	5.69
p	<0.0001	0.048	0.41	<0.0001	<0.0001	<0.0001

Data are average ± SD. Values in the same column with differing superscripts are significantly different ((Fisher's LSD, $p < 0.05$). Rating scale: "extremely dislike" (1) - "extremely like" (9).